1998. The Journal of Arachnology 26:382–384

RESEARCH NOTE

A NEW METHOD OF MARKING SPIDERS

Marking spiders for future identification is essential for many types of ecological and behavioral studies. The perfect marker would not be lost, become unrecognizable, or be transferred to unmarked individuals during the time-frame of the study. The marker, or its application procedure, should not affect health, survivability, or behavior of the individual—including their mobility and catchability. In addition, a simple, rapid protocol with minimal handling is desirable.

However, marking spiders is difficult. A dab of paint applied externally is used commonly, but spiders have little area to paint: the prosoma bears the eyes, the opisthosoma is very flexible, and the joints in legs may seize if an excessive amount of paint is applied. The process usually requires direct handling of the animal, which can be damaging for fragile species without strongly sclerotized exoskeletons. Furthermore, such an external mark will only persist until the exoskeleton is molted.

Histological stains, as a form of internal marking, are an alternative to paints. Several of these have been used in insects (Zacharuk 1963; Barbosa & Peters 1971; Lai et al. 1983; Su et al. 1988, 1991; Oi & Su 1994), and we thought it likely that stains could also be used in spiders. The lightly sclerotized exoskeleton of the opisthosoma in some species could be advantageous as internal staining should be seen more clearly. Further, internal markers should persist between molts. We wanted a simple staining technique that avoided handling the spiders, so we capitalized on their predacious nature and offered them stained prey.

We used termites as the prey and chose to test two non-toxic, fat stains that are suitable as markers in termites, Nile Blue A and Sudan Yellow (Fast Garnet). Two species of termite were used, *Coptotermes lacteus* (Froggatt 1898) were stained blue (0.5% Nile Blue A in distilled water), whereas *Nasutitermes exitiosus* (Hill 1925) were stained pink (4.0% Sudan Yellow dissolved in acetone) (see Evans 1997 for details). The termites were fed stained filter paper for six days, by which time they were colored deeply.

We used *Pholcus phalangioides* (Fuesslin 1775) (Pholcidae) as a test species because of its fragile morphology, translucent exoskeleton and ubiquity. We collected 49 *P. phalangioides* of varying instars from the CSIRO Black Mountain site in Canberra. They were weighed and placed in plastic containers (20 × 15 cm), kept at 28 °C and 90% humidity, sprayed with water, and allowed to weave a web. The spiders were assigned to one of three treatments: blue (i.e., fed blue *C. lacteus*), yellow (i.e., fed pink *N. exitiosus*), and control (i.e., fed unstained *C. lacteus* and *N. exitiosus*). There were no significance differences in initial weight between treatments ($F2, 46 = 1.289$, $P > 0.2$) (Table 1).

After two days, spiders in the two stain treatments were fed 2–5 stained termites, depending on body weight (*ca.* 1 termite per 8 mg of spider body weight), whereas the control spiders were fed similar amounts of unstained termites. Color was clearly visible in the abdomens by the next day: 17 of the 22 spiders in the blue treatment, 8 of the 15 spiders in the yellow treatment. The unstained spiders in those treatments were then fed more stained termites on the second day, which colored them by the third day. The marking was not uniform over the opisthosoma; instead it was most obvious in lighter colored patches and on the ventral surface. This was particularly so in the yellow treatment. We changed the diet to unstained termites once spiders were colored. The spiders captured and ate all termites similarly; regardless of prey color, the termites were always captured and feeding began within five minutes of the termites being dropped into webs. The color in the spiders faded slowly and forwards: the anterior, ventral part of the abdomen remained pink for

Table 1.—Weight and growth (mean ± standard error) of *Pholcus phalangioides* during the five week staining experiment.

Treatment	N	Initial Weight (mg)	Final Weight (mg)	Weight Change Ratio	Number of Molts
Control	12	19.54 ± 3.39	23.0 ± 2.77	1.30 ± 0.08	1.00 ± 0.25
Blue	22	14.94 ± 2.05	18.8 ± 1.78	1.41 ± 0.07	1.00 ± 0.15
Yellow	15	20.11 ± 2.94	23.3 ± 2.20	1.35 ± 0.10	0.67 ± 0.21

around one week (Sudan Yellow) or two weeks (Nile Blue A). Once the color had faded almost completely, we fed the spiders one or two stained termites, which re-colored the spiders.

The experiment concluded after the spiders had been colored and faded three times, over a period of five weeks. There were no deaths, and the spiders grew non-significantly differently during this time. Spiders molted *ca.* once on average in each treatment ($F_{2, 46} = 0.973$, $P > 0.3$), importantly the stain persisted between molts. Spiders had a similar final weight in each treatment ($F_{2, 46} = 1.554$, $P > 0.2$) and had similar growth in each treatment ($F_{2, 46} = 0.426$, $P > 0.6$) (Table 1). Of the eight adult females in the experiment, six were stained (three each blue and pink); and four produced an uncolored eggsac (three blue and one pink). These were carried in the females' chelicerae without any obvious deviation from normal behavior. We did not wait for the eggs to hatch, and so do not know if they were viable.

We concluded from this simple experiment that both histological stains tested in this study do have potential as markers for *P. phalangioides,* and perhaps for other spiders. Although neither marked the spiders permanently, the colors did persist for up to 21 days especially in younger instars at a constant 28 °C and could be reapplied. Importantly, neither stain appeared to affect the behavior or growth of the spiders: webs were destroyed when spiders were removed for weighing, all spiders in all treatments constructed new webs within a day, and weight changes were similar (Table 1). More elaborate laboratory and field trials are necessary to confirm these findings. Perhaps the best aspect of histological stain markers was the marking procedure. It was quick, simple and did not include handling the spiders, thus ensuring an absolute minimum of disturbance to the animal.

The stains tested in this study were not perfect markers as they faded, necessitating remarking. However, remarking was simple and did not appear to affect the spider. Although no spiders died in this study, long term effects may arise from stains applied early in the life cycle (see discussion in Barbosa & Peters 1971 for effects on some insects). Field studies need to address changes in mortality due to predation (e.g., marked individuals may be either attractive or repulsive for their predators). It is also possible that the stains could be transmitted to unmarked spiders, if they successfully invaded the web of and ate the marked individual.

There are other histological stains which have potential as markers. We have also fed *C. lacteus* stained with Neutral Red (0.5% in water) to 12 *P. phalangioides* (mean weight 26.8 g). This colored the spiders purple overnight, with similar variation in the opisthosoma to that described above, persisting for two weeks without apparent harm. Other stains used on termites include Sudan Red 4, Sudan Red 7B and Sudan Black (Su et al. 1988; 1991; see also Conn 1977 for general histological stain information). Other insect species have been marked using histological stains (e.g., beetle larvae, Zacharuk 1963) so these could be used instead of termites as prey. Of course this method of internal marking will only mark those spider species that do not have strong coloring in their exoskeletons. Yet it may be possible to mark lightly colored spiderlings of such species. We hope that other workers can adapt this technique to their species and studies, but after careful assessment of the limitations found or suggested from this study.

We thank A.B. Cady for his comments on the manuscript and Mark Harvey for his help with taxonomic citations.

LITERATURE CITED

Barbosa, P. & T.M. Peters. 1971. The effects of vital dyes on living organisms with special ref-

erence to methylene blue and neutral red. Histochem. J., 3:71–93.
Conn, H. 1977. Biological Stains: A Handbook On The Nature And Uses Of The Dyes Employed In The Biological Laboratory (9th ed). Williams & Wilkins, Baltimore.
Evans, T.A. 1997. Evaluation of marks for Australian subterranean termites (Isoptera: Rhinotermitidae and Termitidae). Sociobiology, 29:1–16.
Lai, P.Y., M. Tamashiro, J.K. Fujii, J.R. Yates & N.Y. Su. 1983. Sudan red 7B, a dye marker for *Coptotermes formosanus*. Proc. Hawaiian Entomol. Soc., 24:277–282.
Oi, F.M. & N.Y. Su. 1994. Stains tested for marking *Reticulitermes flavipes* and *R. virginicus* (Isoptera: Rhinotermitidae). Sociobiology, 24: 241–268.
Su, N.Y., R.H. Scheffrahn & P.M. Ban. 1988. Retention time and toxicity of a dye marker, Sudan red 7B, on Formosan and eastern subterranean termites (Isoptera: Rhinotermitidae). J. Entomol. Science, 23:235–239.
Su, N.Y., P.M. Ban & R.H. Scheffrahn. 1991. Evaluation of twelve dye markers for population studies of the eastern and Formosan subterranean termite (Isoptera: Rhinotermitidae). Sociobiology, 19:349–362.
Zacharuk, R.Y. 1963. Vital dyes for living elaterid larvae. Canadian J. Zool., 41:991–996.

Theodore A. Evans and **Patrick V. Gleeson**: CSIRO Division of Entomology, Canberra, ACT 2601, Australia

Manuscript received 8 May 1997, revised 20 November 1997.